Anti-ITCH Antibody

ER1901-94

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size 102/98/86 kDa.

Description: Acts as an E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates . Involved in the control of inflammatory signaling pathways . Essential component of a ubiquitin-editing protein complex, comprising also TNFAIP3, TAX1BP1 and RNF11, that ensures the transient nature of inflammatory signaling pathways . Promotes the association of the complex after TNF stimulation . Once the complex is formed, TNFAIP3 deubiquitinates 'Lys-63' polyubiquitin chains on RIPK1 and catalyzes the formation of 'Lys-48'-polyubiquitin chains . This leads to RIPK1 proteasomal degradation and consequently termination of the TNF- or LPS-mediated activation of NFKB1. Ubiquitinates RIPK2 by 'Lys-63'-linked conjugation and influences NOD2-dependent signal transduction pathways. Regulates the transcriptional activity of several transcription factors, and probably plays an important role in the regulation of immune response . Ubiquitinates NFE2 by 'Lys-63' linkages and is implicated in the control of the development of hematopoietic lineages .

Immunogen: Recombinant protein within Human ITCH aa 302-481 / 903.

Positive control: Siha cell lysates, A431 cell lysates, Mouse spleen tissue lysates, Mouse pancreatic tissue lysates, Hela, LOVO, Siha, Human liver cancer tissue, rat brain tissue, mouse pancreas tissue.

Subcellular location: Cell membrane, Cytoplasm, Endosome, Membrane, Nucleus.

Database links: SwissProt: Q96J02 Human | Q8C863 Mouse | A0A8I5ZZU1 Rat

Recommended Dilutions:

WB	1:1000-1:5000
IHC-P	1:50-1:200
IF-Cell	1:100-1:500
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

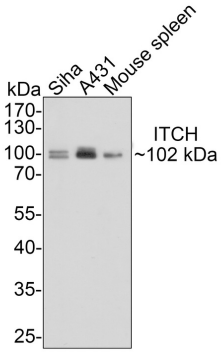

Fig1: Western blot analysis of ITCH on different lysates with Rabbit anti-ITCH antibody (ER1901-94) at 1/500 dilution.

Lane 1: Siha cell lysate
Lane 2: A431 cell lysate
Lane 3: Mouse spleen tissue lysate (20 µg/Lane)

Lysates/proteins at 10 µg/Lane.

Predicted band size: 102/98/86 kDa.
Observed band size: 102 kDa

Exposure time: 2 minutes;

8% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1901-94) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

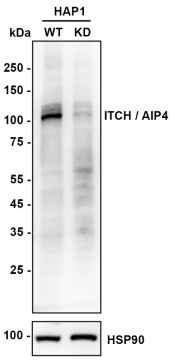

Fig2: Western blot analysis of ITCH on different lysates with Rabbit anti-ITCH antibody (ER1901-94) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-ITCH KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 102 kDa
Observed band size: 102 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1901-94) at 1/2,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

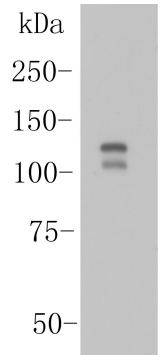

Fig3: Western blot analysis of ITCH on Mouse pancreatic tissue lysate. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ER1901-94, 1/1000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

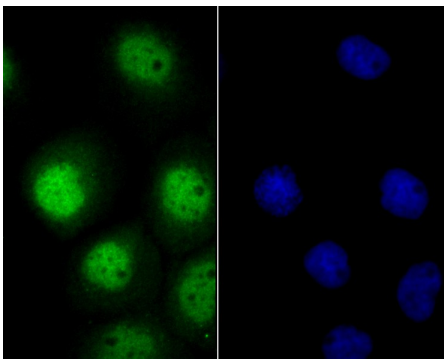

Fig4: ICC staining of ITCH in A431 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ER1901-94, 1/200) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

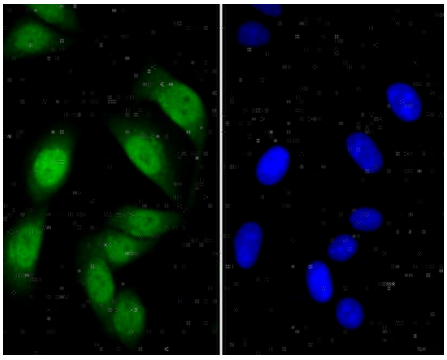

Fig5: ICC staining of ITCH in Siha cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ER1901-94, 1/200) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

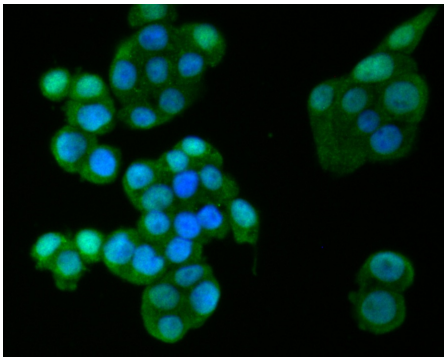

Fig6: ICC staining of ITCH in LOVO cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ER1901-94, 1/200) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

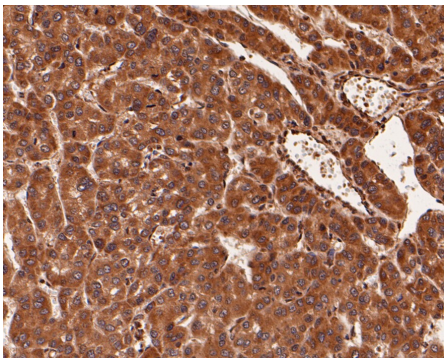

Fig7: Immunohistochemical analysis of paraffin-embedded Human liver cancer tissue using anti-ITCH antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-94, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

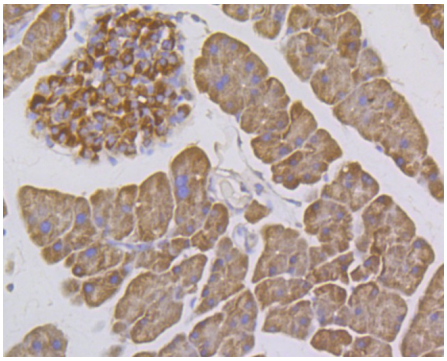

Fig8: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue using anti-ITCH antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1901-94, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

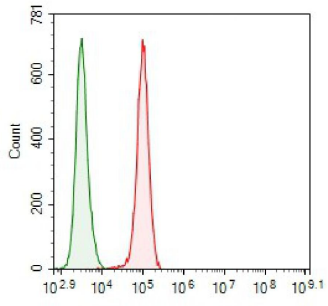

Fig9: Flow cytometric analysis of ITCH was done on LOVO cells. The cells were fixed, permeabilized and stained with the primary antibody (ER1901-94, 1/100) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; green).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Marchese A.et.al.The E3 ubiquitin ligase AIP4 mediates ubiquitination and sorting of the G protein-coupled receptor CXCR4.Dev. Cell 5:709-722(2003).